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THE YIELD OF ENERGY TRANSFER AND THE SPECTRAL DISTRIBUTION OF EXCITATION ENERGY IN THE PHOTOCHEMICAL APPARATUS OF FLASHED BEAN LEAVES

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SUMMARY

The distribution of excitation energy in the photochemical apparatus of photosynthesis was examined with dark-grown bean leaves which had been partially greened by a series of brief xenon flashes. The yield of energy transfer from Photosystem II to Photosystem I was estimated by a method devised recently for spinach chloroplasts. Using that method, which involves a comparison of fluorescence excitation spectra with the absorption spectrum of the flashed leaves at −196 °C, the yield of energy transfer in the flashed leaves was found to be about twice the yield values obtained with chloroplasts. On further greening in continuous light, during which time the light-harvesting chlorophyll *a*/*b* protein accumulates, the yield of energy transfer decreases approaching the lower values found previously for spinach chloroplasts. The initial distribution of absorbed quanta between Photosystems I and II was also determined for the flashed leaves as a function of the wavelength of excitation. In contrast to spinach chloroplasts or to mature green leaves where that distribution is almost constant independent of wavelength at wavelengths shorter than 675 nm, the distribution of energy in the flashed leaves showed considerable variation with wavelength. The rate of photooxidation of *P*-700 at −196 °C was measured at two wavelengths, 645 and 670 nm, at which the initial distribution of energy between the photosystems is appreciably different. A comparison of the rates of photooxidation of *P*-700 with the relative intensities of Photosystem I fluorescence excited by these two wavelengths confirmed that energy was transferred from Photosystem II to Photosystem I and that the transferred energy stimulated both the photooxidation of *P*-700 and Photosystem I fluorescence at −196 °C.

INTRODUCTION

In a recent study [1] the model of the photochemical apparatus of photosynthesis proposed by Butler and Kitajima [2, 3] was expanded to show that the yield of energy transfer from Photosystem II to Photosystem I, $\varphi_{T(II \to I)}$, could be obtained from a comparison of fluorescence excitation spectra with the absorption spectrum

measured on individual samples of chloroplasts frozen to −196 °C. The results of that study were in good agreement with earlier determinations of $\varphi_{T(II\rightarrow I)}$ made from measurements of the fluorescence of chloroplasts suspended in the presence and absence of Mg^{2+} [4].

According to the model, the 730 nm emission band from chloroplasts at −196 °C emanates from the antenna chlorophyll of Photosystem I but that fluorescence can be excited by either the direct excitation of Photosystem I or by the initial excitation of Photosystem II with subsequent energy transfer to Photosystem I. The intensity of Photosystem I fluorescence at 730 nm at −196 °C can be expressed as:

$$F_I = (\alpha+\beta\varphi_{T(II\rightarrow I)})I_a\varphi_{FI} \quad (1)$$

where α is the fraction of quanta absorbed by the photochemical apparatus which is distributed initially to Photosystem I, β is the remaining fraction which is distributed to Photosystem II, I_a is the quantum flux absorbed by the photochemical apparatus and φ_{FI} is the probability that an excited Photosystem I chlorophyll molecule will fluoresce.

It was recognized [2] that $\varphi_{T(II\rightarrow I)}$ varies from a minimum value, $\varphi_{T(II\rightarrow I)(o)}$, when the Photosystem II reaction centers are all open to a maximum value, $\varphi_{T(II\rightarrow I)(M)}$, when the Photosystem II centers are all closed to the same relative extent (and for the same reason) that the yield of Photosystem II fluorescence increases as the Photosystem II reaction centers close. Thus, according to Eqn. 1, the fluorescence from Photosystem I will also vary from a minimum $F_{I(o)}$ level to a maximum $F_{I(M)}$ level due to the dependence of $\varphi_{T(II\rightarrow I)}$ on the state of the Photosystem II reaction centers. However, photochemical changes associated with Photosystem I activity at −196 °C do not result in any fluorescence yield changes in chloroplasts [5] or in flashed leaves [6]. In red algae where the photooxidation of *P*-700 does cause an increase in the yield of Photosystem I fluorescence at −196 °C [7], Eqn. 1 is still valid but in that case φ_{FI} depends on the state of the Photosystem I reaction centers.

It is apparent from Eqn. 1 that the fluorescence from Photosystem I has two sources of excitation so that the excitation spectrum for Photosystem I fluorescence at the $F_{I(M)}$ level can be treated as the sum of two excitation spectra:

$$F_{I(M)} = F_{I(\alpha)}+F_{I(\beta)} \quad (2)$$

where from Eqn. 1 $F_{I(\alpha)} = \alpha I_a\varphi_{FI}$ and $F_{I(\beta)} = \beta\varphi_{T(II\rightarrow I)(M)}\, I_a\varphi_{FI}$. These two excitation spectra, one representing the direct excitation of Photosystem I, the other, indirect excitation via energy transfer from Photosystem II, can be determined from measurements of fluorescence at −196 °C [1].

It was shown in the previous paper [1] from the equations of the model that:

$$F_{I(\alpha)} + \frac{F_{I(\beta)}}{\varphi_{T(II\rightarrow I)(M)}} = I_a\varphi_{FI} \quad (3)$$

Once the excitation spectra for $F_{I(\alpha)}$ and $F_{I(\beta)}$ are known, the method of determining $\varphi_{T(II\rightarrow I)(M)}$ involves fitting the absorption spectrum, I_a (measured on a linear photometric scale), with a weighted sum of the fluorescence excitation spectra, $F_{I(\alpha)}+KF_{I(\beta)}$, and taking the value of K which gives the best fit to be the reciprocal of $\varphi_{T(II\rightarrow I)(M)}$. In these procedures it is important to bear in mind the distinction between the absorption spectrum of the photochemical apparatus and the absorption spectrum

of the sample. Any pigment molecules which do not transfer their excitation energy to chlorophyll are not considered to be a part of the photochemical apparatus. Thus, in the blue region I_a may be smaller than the total quantum flux absorbed because some of the energy absorbed by carotenoid pigments is not transferred to chlorophyll. However, at wavelengths longer than 580 nm it is assumed that all of the energy is absorbed by chlorophyll and that all of the chlorophyll is a part of the photochemical apparatus so I_a is taken to be identical to the absorption spectrum of the sample. The curve fitting procedures are employed only in this longer wavelength region of the visible spectrum.

It was also shown previously [1] that the wavelength dependence of α can be determined from these excitation spectra once the value of $\varphi_{T(II\to I)}$ is known.

$$\alpha = \frac{F_{I(\alpha)}}{F_{I(\alpha)} + F_{I(\beta)}/\varphi_{T(II\to I)(M)}} \tag{4}$$

In these equations $F_{I(\alpha)}$, $F_{I(\beta)}$, α, β, and I_a are all functions of wavelength and can be treated as spectral distributions.

The spectral dependence of the energy distribution to Photosystem I and Photosystem II can be specified in terms of these parameters. The initial energy flux distributed to Photosystem I and Photosystem II can be represented by the spectra for $\alpha\, I_a$ and β_a, respectively. However, the wavelength dependence of the energy actually available to Photosystem I and Photosystem II will be represented by the spectral distributions of $(\alpha+\beta\varphi_{T(II\to i)})I_a$ and $(\beta-\beta\varphi_{T(II\to I)})I_a$ which depend on the state of the Photosystem II reaction centers.

The purpose of the work reported here was to determine the yield of energy transfer from Photosystem II to Photosystem I and the wavelength dependence of α in flashed leaves which lack the light-harvesting chlorophyll *a*/*b* protein. The results show appreciably higher yields of energy transfer and a greater wavelength dependence for α than were reported previously [1] with spinach chloroplasts.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris* var. red kidney) were soaked overnight and planted in vermiculite. After 4 days in darkness the plants were irradiated with a repetitive series of 1 ms flashes from a xenon lamp once every 12 min for 6 days. All measurements were carried out on leaf samples in the form of a 1 cm disc frozen to liquid nitrogen temperature in our vertical cuvette and Dewar system [8].

The fluorescence emission spectrum (corrected for the spectral response of the phototube) was measured with the fiber optics arrangement described previously [6]. Fluorescence excitation spectra were measured with our computer-linked, single-beam spectrophotometer [8]. For fluorescence excitation spectra, the monochromatic exciting light was transmitted from the Cary Model 14 monochromator (0.4-mm slits) to the top of the frozen sample with a fiber optic light-pipe assembly which also transmitted the fluorescence from the top of the sample back to the phototube of the spectrophotometer. Fluorescence was measured either at 694 nm (defined by a filter system consisting of a Baird Atomic 694 nm interference filter with Corning 9830 and 2030 glass filters) or at 730 nm (Baird Atomic 730 nm interference filters with Corning 2600 and 5031 filters). The passband of the monochromatic exciting light was approx.

1.5 nm and the intensity of excitation was so low that the measurement of the excitation spectra at the minimum, F_o, level did not alter the fluorescence yield of the frozen sample. Fluorescence excitation spectra were measured at the F_o level and the F_M level (obtained after a saturating irradiation with blue light). The fluorescence excitation spectra are corrected for equal quantum flux.

The absorption spectrum of the frozen sample was measured on a linear photometric scale. The transmittance of the sample, T, was measured as the ratio of photocurrent obtained with the frozen sample to that obtained with a frozen buffer blank and the absorption spectrum, I_a, was plotted as $(1-T)$.

The computer was used to calculate difference spectra or ratio spectra between various pairs of spectra or to multiply a spectrum by a constant or to take the first derivative of a spectrum. The spectra presented in the figures of this paper were all plotted directly from the computer from data measured on line with the spectrophotometer.

The rate of photooxidation of *P*-700 was measured by the repetitive pulse method employed previously [6] except that pulses of monochromatic light (0.1-s pulses at 1 Hz) were used instead of the xenon flashes. The monochromatic light was defined by 645 and 670 nm interference filters and the two wavelengths of excitation could be interchanged rapidly. The intensities of the 645 and 670 nm excitation beams were 0.80 and 0.77 mW/cm^2 at the sample.

RESULTS AND DISCUSSION

The emission spectrum of a flashed leaf at the F_M level at −196 °C is shown in Fig. 1. The spectrum shows the emission bands of the antenna chlorophyll *a* of Photosystem I and Photosystem II at 730 and 694 nm, respectively. However, the 685 nm band, wnich is found in the low temperature emission spectra of mature chloroplasts, is absent, presumably because the flashed leaves lack the light-harvesting chlorophyll *a*/*b* protein. The different categories of fluorescence which we distinguish and use in our analysis of energy distribution are also shown schematically in Fig. 1. Irradiating a

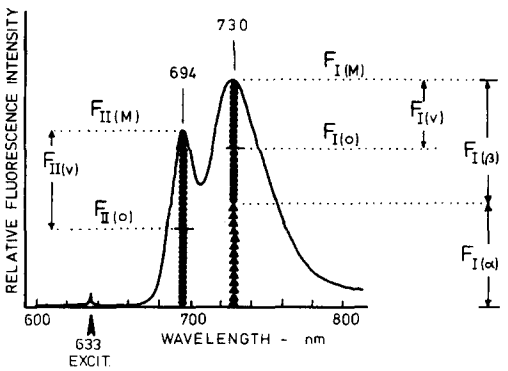

Fig. 1. Emission spectrum of a flashed leaf at −196 °C at the F_M level excited by 633 nm light. Intensities of fluorescence at the F_o level at 694 and 730 nm are also indicated. The catagories of fluorescence excited by Photosystem II are indicated by ●, the category of fluorescence excited by Photosystem I is indicated by ▲.

thoroughly dark-adapted leaf at −196 °C causes the 694 nm fluorescence of Photosystem II to increase from the $F_{II(o)}$ level to $F_{II(M)}$; likewise the 730 nm fluorescence of Photosystem I increases from $F_{I(o)}$ to $F_{I(M)}$. Those increases of fluorescence between the F_o and F_M levels define the fluorescence of variable yield, F_V, for Photosystem I and Photosystem II fluorescence. In addition, the separation of $F_{I(M)}$ into $F_{I(\alpha)}$ and $F_{I(\beta)}$, as discussed in Introduction, is shown in the diagram. The intensities of the different categories of fluorescence at 694 and 730 nm are indicated by two symbols. The circles indicate those emissions which have a Photosystem II excitation spectrum while the triangles indicate the emission which has a Photosystem I excitation spectrum. According to our model $F_{I(V)}$ and $F_{I(\beta)}$ are excited by an initial absorption of quanta by Photosystem II with subsequent energy transfer to Photosystem I.

Fluorescence excitation spectra were measured at the F_o and F_M levels from a flashed leaf at −196 °C for emission at 730 nm (Fig. 2) and emission at 694 nm (Fig. 3). Excitation spectra for the fluorescence of variable yield, F_V, were computed as the difference spectrum $F_M - F_o$ at the two emission wavelengths. According to our model the fluorescence of variable yield at 730 nm, $F_{I(V)}$, should have a pure Photosystem II excitation spectrum. Also, all of the fluorescence, F_M, F_o and F_V, measured at 694 nm should have Photosystem II excitation spectra. It is apparent that all of the excitation spectra in Fig. 3 are very similar. That similarity is confirmed by the wavelength dependence of the ratio $F_{II(V)}/F_{II(M)}$ shown in the upper part of Fig. 3. The excitation spectrum of $F_{I(V)}$ shown in Fig. 2 is appreciably different from the other Photosystem I excitation spectra, $F_{I(o)}$ and $F_{I(M)}$, but it is very similar to the Photosystem II excitation spectra presented in Fig. 3. That similarity is established by the wavelength dependence of the ratio $F_{I(V)}/F_{II(V)}$, also in the upper part of Fig. 3.

It was shown previously [1] that the excitation spectrum for that part of F_I which is due to direct excitation via α can be calculated from the fluorescence excitation spectra which are presented in Figs. 2 and 3.

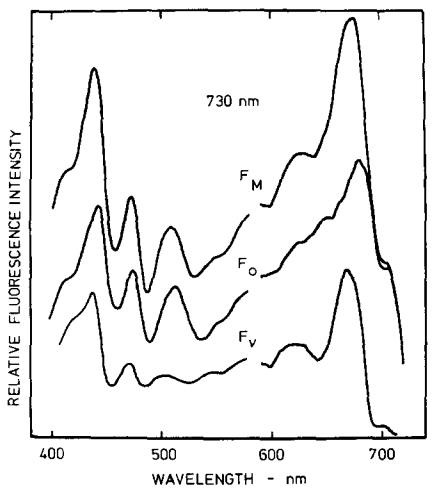

Fig. 2. Excitation spectra for 730 nm fluorescence from a flashed leaf at −196 °C. The spectra were measured at the F_o and F_M levels in two separate scans as indicated. The excitation spectrum for F_V was calculated as the difference spectrum $F_M - F_o$.

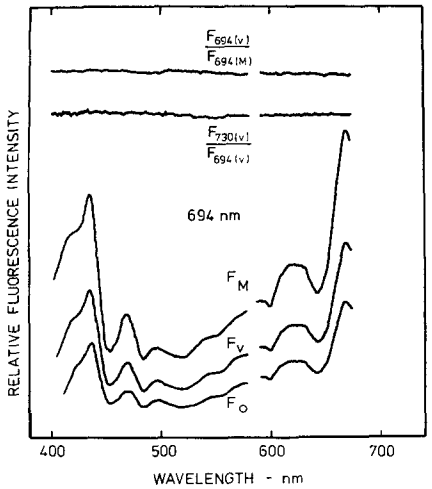

Fig. 3. Excitation spectra for 694 nm fluorescence from a flashed leaf at −196 °C. The spectra were measured at the F_o and F_M levels and the excitation spectrum for F_V was obtained as the difference spectrum $F_M - F_o$. Ratio of the excitation spectra $F_{690(V)}/F_{694(M)}$ and of $F_{730(V)}$ (from Fig. 2)/$F_{690(V)}$ were calculated and plotted by the computer.

$$F_{I(\alpha)} = F_{I(M)} - \frac{F_{II(M)}}{F_{II(V)}} F_{I(V)} \tag{5}$$

This calculation (which for any given wavelength of excitation represents the extrapolation of the straight-line plot of F_{730} vs. F_{694} between the F_M and F_o levels back to the F_{730} axis [5, 6]) requires that the three categories of fluorescence which comprise the last term in Eqn. 5 all be due to Photosystem II excitation. That requirement is established for the flashed leaves in Fig. 3. The experimental value of the ratio $F_{II(M)}/F_{II(V)}$, which will be needed for Eqn. 5, is 1.8 for the flashed leaf at −196 °C.

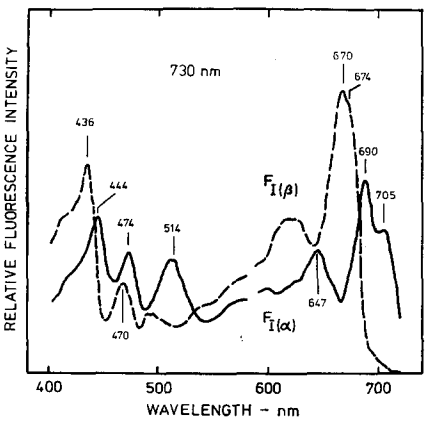

Fig. 4. Excitation spectra for $F_{I(\alpha)}$ and $F_{I(\beta)}$ of a flashed leaf at −196 °C calculated by the computer using the fluorescence excitation spectra in Figs. 2 and 3 and the equations in the text.

Thus, using the excitation spectra for $F_{I(M)}$ and $F_{I(V)}$ shown in Fig. 2 and the value of 1.8 for the ratio $F_{II(M)}/F_{II(V)}$, according to Eqn. 5, $F_{I(\alpha)}$ can be plotted as the difference spectrum $F_{I(M)} - 1.8\ F_{I(V)}$. Then, given the excitation spectrum for $F_{I(\alpha)}$, $F_{I(\beta)}$ can be plotted according to Eqn. 2 as the difference spectrum $F_{I(M)} - F_{I(\alpha)}$. The excitation spectra for $F_{I(\alpha)}$ and $F_{I(\beta)}$ are shown in Fig. 4.

It is apparent from differentiating Eqn. 3 with respect to λ:

$$\frac{dF_{I(\alpha)}}{d\lambda} + \frac{1}{\varphi_{T(II\to I)(M)}} \frac{dF_{I(\beta)}}{d\lambda} = \varphi_{FI} \frac{dI_a}{d\lambda} \tag{6}$$

that at the wavelength maximum of I_a where $dI_a/d\lambda = 0$:

$$\varphi_{T(II\to I)(M)} = -\frac{dF_{I(\beta)}/d\lambda}{dF_{i(\alpha)}/d\lambda} \tag{7}$$

The absorption spectrum of a flashed leaf at −196 °C from 580 to 720 nm, measured on a linear photometric scale, is shown as the dotted curve, I_a, in Fig. 6. The first derivative spectrum of I_a is shown in Fig. 5 along with the first derivative spectra for $F_{I(\alpha)}$ and $-F_{I(\beta)}$. At 672 nm where $dI_a/d\lambda = 0$, we estimate from the data in Fig. 5 that $-dF_{I(\beta)}/d\lambda$ is 24 relative units and that $dF_{i(\alpha)}/d\lambda$ is 38 relative units so that we calculate from Eqn. 7 that $\varphi_{T(II\to I)(M)}$ is 24/38 or 0.63. The excitation spectrum for $F_{I(\beta)}$ shown in Fig. 4 was multiplied by 1.6, the reciprocal of 0.63, and added to the excitation spectrum of $F_{I(\alpha)}$, also shown in Fig. 4, and that summation spectrum, $F_{I(\alpha)} + 1.6\ F_{I(\beta)}$, was compared against the absorption spectrum, I_a, according to Eqn. 3, with both spectra being normalized at the 672 nm maximum. The comparison between those two spectra shown in Fig. 6 is quite good except at wavelengths longer than 700 nm where C-705 absorbs. C-705 is present in the flashed leaves and, as was the case with spinach chloroplasts [1], the direct excitation of C-705 at −196 °C results in a higher yield of

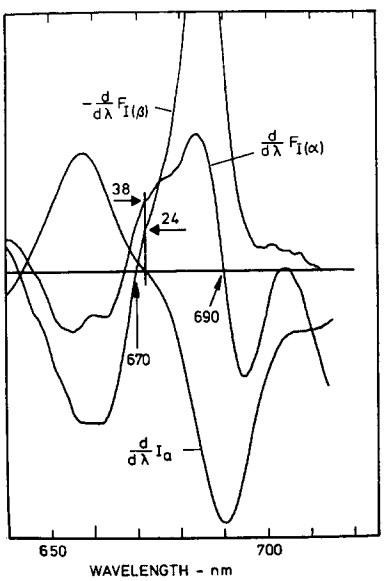

Fig. 5. First derivative spectra of $F_{I(\alpha)}$, $-F_{I(\beta)}$ and I_a calculated and plotted by the computer.

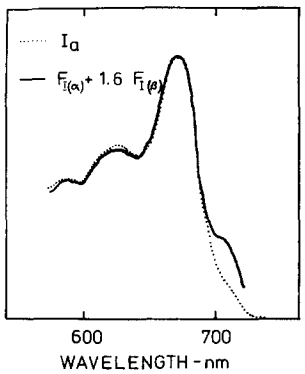

Fig. 6. Absorption spectrum I_a (measured on a linear photometric scale, $1-T$) of a flashed leaf at −196 °C (. . . . .); weighted sum of fluorescence excitation spectra, $F_{I(\alpha)} + 1.6\ F_{I(\beta)}$ (−).

730 nm fluorescence than when excitation is via the shorter wavelength-absorbing forms of chlorophyll.

The yield of energy transfer from Photosystem II to Photosystem I increases from a minimum value when the Photosystem II reaction centers are open to a maximum value when the Photosystem II centers are closed to the same relative extent that the yield of Photosystem II fluorescence increases. The value of 1.8 for the ratio $F_{II(M)}/F_{II(V)}$ translates to a value of 2.2 for the ratio $F_{II(M)}/F_{II(o)}$. Thus we estimate $\varphi_{T(II\to I)(o)}$ for the flashed leaves to be 0.28.

The value of $\varphi_{T(II\to I)(M)}$ was determined with the flashed leaves after various times of greening in continuous white light. The results presented in Table I show that the value of $\varphi_{T(II\to I)(M)}$ decreases with time of greening until, after 6 h, it reaches the values which are characteristic for mature chloroplasts.

TABLE I

$\varphi_{T(II\to I)}$ DURING FURTHER GREENING OF FLASHED LEAVES IN CONTINUOUS WHITE LIGHT

	Continuous illumination (h)				
	0	0.5	1.0	2.0	6.0
$\varphi_{T(II\to I)(M)}$	0.61	0.50	0.42	0.35	0.28
$\varphi_{T(II\to I)(o)}$	0.28	0.23	0.19	0.16	0.13

Given the excitation spectra for $F_{I(\alpha)}$ and $F_{I(\beta)}$ (Fig. 4) and the value of $\varphi_{T(II\to I)(M)}$ for the flashed leaves, the wavelength dependence of α was calculated by the computer according to Eqn. 4. These results for the wavelength dependence of α, shown in Fig. 7, are markedly different from the results obtained previously with spinach chloroplasts. With chloroplasts α was essentially constant at a value of 0.3 from 400 to 675 nm but increased sharply toward unity at longer wavelengths as Photosystem I became the dominant absorber. With the flashed leaves α shows marked variations in the blue, indicating that some carotenoid pigment molecules associated with the photochemical apparatus transfer their excitation energy preferentially to Photo-

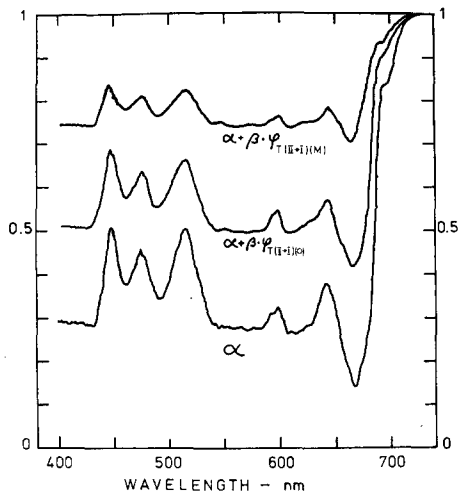

Fig. 7. Wavelength distribution of α of a flashed leaf at -196 °C (calculated from Eqn. 4 in the text). The spectral distribution of $\alpha+\beta\varphi_{T(II\to I)(o)}$ and $\alpha+\beta\varphi_{T(II\to I)(M)}$ are also shown for $\varphi_{T(II\to I)(o)} = 0.28$ and $\varphi_{T(II\to I)(M)} = 0.63$.

system I. In the longer wavelength portion of the spectrum α has a minimum value of about 0.15 at 670 nm, a nearly maximum of about 0.40 at 640 nm. The constancy of α in chloroplasts was attributed to the presence of the large amount of the light-harvesting chlorophyll *a*/*b* protein which would tend to buffer out the variations in α which would otherwise arise from the differences between the absorption spectra of Photosystem I and Photosystem II. As expected, the flashed leaves, which lack the light-harvesting chlorophyll *a*/*b* protein, show an appreciable variation of α with wavelength. α indicates the initial distribution of quanta to Photosystem I. The wavelength dependence of the energy available to Photosystem I, taking into account the energy transferred from Photosystem II, is shown in Fig. 7 by the curve $\alpha+\beta\varphi_{T(II\to I)(o)}$, for the case where the Photosystem II reaction centers are all open, and by the curve $\alpha+\beta\varphi_{T(II\to I)(M)}$ for the case where the Photosystem II centers are all closed.

In chloroplasts the excitation spectra for $F_{I(M)}$, $F_{I(\alpha)}$ and $F_{I(\beta)}$ were all essentially the same from 400 to 675 nm because α was essentially constant over this wavelength range [5]. In flashed leaves the excitation spectra for $F_{I(\alpha)}$ and $F_{I(\beta)}$ are markedly different from each other and from the excitation spectra found with chloroplasts or mature green leaves. On greening, $F_{I(\alpha)}$ rapidly develops an excitation band at 680 nm which becomes the dominant excitation. At the same time, in $F_{I(\beta)}$, the 674 nm excitation band increases and an excitation band at 650 nm develops as chlorophyll *b* accumulates. Also, as the light-harvesting chlorophyll *a*/*b* protein accumulates, the differences between these excitation spectra of $F_{I(\alpha)}$ and $F_{I(\beta)}$ diminish. The final result after greening is that the two excitation spectra representing Photosystem I and Photosystem II activity become remarkably similar for wavelengths shorter than 675 nm [1].

It was recognized in the original descriptions of the model of the photochemical apparatus that the conclusion that F_V at 730 nm represented energy transfer from Photosystem II to Photosystem I depended on the assumption that a large fraction of

the 730 nm fluorescence at −196 °C was due to Photosystem I. Low temperature emission spectra of highly enriched Photosystem II particles supported that assumption [4, 9] but we could not rule out the possibility that the chlorophyll of Photosystem II in vivo had a long wavelength emission band at −196 °C which accounted for the F_V that was observed at 730 nm. If that were the case our conclusions about energy transfer from Photosystem II to Photosystem I would be in error. Thus, more direct manifestations of energy transfer were sought. It was found that Photosystem I activity, assayed either as electron transfer rates at room temperature or as the rate of photooxidation of *P*-700 at −196 °C [6, 10], was stimulated when the Photosystem II reaction centers were closed, presumably because the yield of energy transfer from Photosystem II to Photosystem I increased as the Photosystem II centers closed. These measurements were taken to be a direct indication of energy transfer and a confirmation of the conclusion that F_V at 730 nm measured at −196 °C was a manifestation of energy transfer from Photosystem II to Photosystem I. The results of the present work on the flashed leaves offer another opportunity to test that conclusion. If there were no energy transfer from Photosystem II to Photosystem I the wavelength dependence for the rate of oxidation of *P*-700 at −196 °C should follow the excitation spectrum of $F_{I(\alpha)}$ (shown in Fig. 4). On the other hand if there is energy transfer to Photosystem I and all of the fluorescence at 730 nm at −196 °C represents emission from Photosystem I, then the wavelength dependence of *P*-700 photooxidation should follow the excitation spectrum of $F_{I(o)}$ (shown in Fig. 2). Such a test is feasible with the flashed leaves because the excitation spectra for $F_{I(\alpha)}$ and $F_{I(o)}$ are sufficiently different. The rate of photooxidation of *P*-700 in a flashed leaf at −196 °C was measured with 645 nm excitation and with 670 nm excitation of equal quantum flux. If the excitation spectrum for $F_{I(\alpha)}$ reflects the wavelength distribution for all of the energy reaching Photosystem I, the rate of oxidation of *P*-700 should be less with the 670 nm excitation. However, if the excitation spectrum for $F_{I(o)}$ indicates that energy distribution, then the rate of *P*-700 oxidation should be considerably larger with 670 nm light than with 645 nm excitation. The results of a typical experiment are shown in Fig. 8. Both rates were obtained from the same sample in order to avoid variations between different frozen leaves. Thus, the experiment was started with 645 nm excitation and then changed to the 670 nm excitation after the transformation was only partially completed. It was found previously [6, 10] that the phototransformation of *P*-700 follows closely to second-order reaction kinetics (screening of the excitation light by the relatively dense samples makes the first-order photoreaction more second order in character). It is apparent from the data in Fig. 8 that the rate of transformation is appreciably greater with the 670 nm excitation. The same result is obtained if the 670 nm light is used first but the difference between the two wavelengths of excitation is more apparent in the raw data if the faster rate follows the slower rate. These measurements were repeated a number of times with flashed leaves and in all cases the rate with 670 nm excitation was approximately twice the rate obtained with an equal quantum flux of 645 nm light. Given the fact that there is energy transfer from Photosystem II to Photosystem I, that yield of that transfer will increase as the Photosystem II reaction centers close. Thus, in Fig. 8, the rate of oxidation of *P*-700 in 670 nm light will be somewhat greater because the Photosystem II reaction centers are partially closed. This effect is relatively small, however, because the rate of oxidation of *P*-700 at −196 °C is considerably faster than the rate of closure of the Photosystem

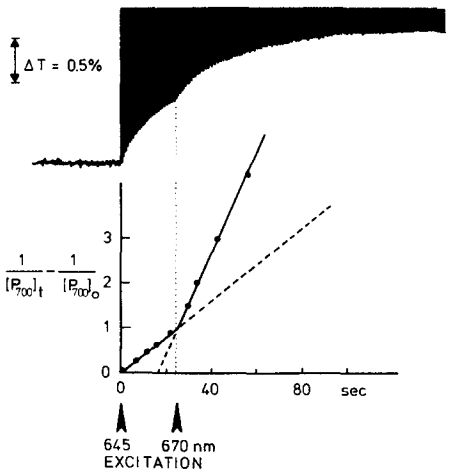

Fig. 8. Photooxidation of *P*-700 in a flashed leaf at −196 °C by pulses of monochromatic light at 645 and 670 nm of equal quantum flux. Lower curves: transformation of *P*-700 plotted by second-order kinetics. Excitation with 645 nm pulses starts at $t = 0$. Excitation changed to 670 nm pulses where indicated.

II centers. In a separate experiment (data not shown) the rate of oxidation of *P*-700 and the rate of increase of Photosystem II fluorescence by the repetitive flashes were measured simultaneously. We estimate from such an experiment that at the time that the actinic light was changed from 645 to 670 nm in Fig. 8, approx. 20 % of the Photosystem II reaction centers were closed. This would cause the rate of photooxidation of *P*-700 to increase by approx. 15 %. Thus, we conclude that the wavelength dependence of the photooxidation of *P*-700 follows reasonably closely to excitation spectrum for 730 nm fluorescence, that some of the energy absorbed by Photosystem II is transferred to Photosystem I and that the transferred energy stimulates both the excitation of fluorescence at 730 nm as well as the photooxidation of *P*-700. This comparison between the action spectrum for *P*-700 oxidation and the fluorescence excitation of 730 nm fluorescence confirms the validity of the original assumption that energy transfer from Photosystem II to Photosystem I does occur and that the fluorescence of variable yield at 730 nm at −196 °C is a manifestation of such energy transfer.

While we conclude from the results of Fig. 8 that a major part of the fluorescence of variable yield at 730 nm is due to energy transfer from Photosystem II, we cannot rule out the possibility that a small part of the fluorescence at 730 m is due to emission from Photosystem II. In fact, we would estimate from the emission spectra of highly enriched Photosystem II particles [9] and from the results presented in the accompanying paper [11] that the emission from Photosystem II at 730 nm could be 10–15 % of the peak emission at 694 nm. This would mean in Fig. 1 that 8–12 % of the emission at 730 nm is due to Photosystem II. While such an overlap is relatively small its effect is not negligible. If we were to correct our value of $\varphi_{T(II\to I)(M)}$ to take that overlap into account the value of 0.63 would be changed to a value of 0.54 assuming the 8 % overlap or to a value of 0.49 assuming the 12 % overlap. It is reasonable to assume the presence of such a long wavelength tail of the Photosystem II fluorescence.

Thus, we would estimate that the yield of energy transfer in the flashed leaves varies from a minimum value of about 0.23 when the Photosystem II reaction centers are all open to a maximum value of about 0.50 when the reaction centers are all closed, values which are about twice those found with mature chloroplasts.

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